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CARDIOVASCULAR

~~RENEWAL~~ Continuation

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: 5/24/74

1. Principal Investigator (give title and degrees):

Theodore A. Slotkin, Ph.D.
Assistant Professor of Pharmacology

2. Institution & address:

Duke University Medical Center
Durham, North Carolina 27710

3. Department(s) where research will be done or collaboration provided:

Department of Physiology and Pharmacology

4. Short title of study:

Catecholamine Stores in Normal and Hypertensive Rats

5. Proposed starting date: Jan. 1, 1975

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

In the previous 18 months of this project we have demonstrated the feasibility of studying sympatho-adrenal amine stores in the hypertensive rat (SHR) and during development in normal rats and have identified specific changes in synthesis, uptake, storage and secretion of catecholamines. During the time period outlined, we hope to:

1. Examine the effects of antihypertensive drugs in the SHR, including reserpine, aldomet, DBH inhibitors, α-methyl-p-tyrosine and 6-hydroxydopamine.
2. Examine the effects of acute and chronic nicotine administration in the SHR; nicotine is reputed to produce prolonged alterations in catecholamine turnover.
3. Detail the development of the adrenal medulla in the SHR to determine whether blood pressure changes precede or follow the alterations we have identified in catecholamine disposition.

8. Brief statement of working hypothesis:

Studies from our and other laboratories have shown that catecholamine disposition is altered in the SHR, but do not indicate conclusively whether this is a causal factor in hypertension. By determining whether the sympatho-adrenal defects appear before or after the onset of hypertension (maturation studies) we may obtain an answer to this question.

We have demonstrated that the effects of drugs which act on storage vesicles are altered in the SHR. These factors may contribute to similar difficulties observed in the therapy of human essential hypertension with these agents. The proposed study will identify how the actions differ and may suggest alternative therapies.

The sympatho-adrenal system of the SHR is set on a "hair trigger" and hyperresponds to small stress inputs. This suggests that chronic administration of nicotine may produce profound alterations in catecholamine stores of the SHR, which in turn could markedly affect cardiovascular function.

9. Details of experimental design and procedures (append extra pages as necessary)

see appended pages 11 - 16

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Laboratory consists of 850 square feet fitted with standard laboratory type benches. Major items of equipment include: Sorvall RC-2B centrifuge, Beckman L5-50 ultracentrifuge and rotors, Farrand ratio fluorometer, catecholamine autoanalyzer, Wang 600-6-TP programmable calculator, incubation baths, pH meter, balances and other general items of laboratory hardware. Research facilities include animal rooms, cold rooms and a spectrophotometer.

11. Additional facilities required:

Funds are requested to purchase a Beckman liquid scintillation counter. We have previously made use of a counter in another department, but as all our assays are radiochemical, it has become increasingly difficult to borrow enough counter time to accomodate our needs. This situation has hampered our ability to obtain results in time to plan future experiments on schedule.

12. Biographical sketches of investigator(s) and other professional personnel (append):

See pages 6, 7, 8 and 9.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See page 10.

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Theodore Slotkin - Principal Investigator		REDACTED
Technical		REDACTED
Frederic J. Seidler (B.S.)		
Fringe Benefits @ 12.45%		
Sub-Total for A		REDACTED
B. Consumable supplies (by major categories)		
Animals, including housing & shipping costs		2500
Isotopes		1500
Chemicals and Hardware		1000
Sub-Total for B		5000
C. Other expenses (itemize)		
Equipment maintenance and service		500
Travel		500
Sub-Total for C		1000
Running Total of A + B + C		$14097
D. Permanent equipment (itemize)		
Beckman liquid scintillation counter (see item 11 on page 3)		8300
Sub-Total for D		8300
E. Indirect costs (16% of A+B+C)		2115
Total request		$24,512

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	8583	5000	1000	0	2188	16,771
Year 3	9098	5000	1000	0	2265	17,363

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Amine stores of developing normal & hypertensive rats*	American Heart Association #73-611	$50,000	10/1/73 - 9/30/76
2. Effects of morphine on the adrenal medulla†	NIMH, DA-00465	85,000	6/1/73 - 5/31/76
3. Faculty development award§	Pharmaceutical Manufacturers Association Foundation	40,000	7/1/73 - 6/30/75

* This project is directed primarily toward maturation studies and funds are mostly for salary support and pregnant rats. The current request is mandatory for continuation of studies on effects of reserpine, nicotine, etc. in adult SHR and to offset the rising costs of working with SHR. For example, one SHR rat costs $7.50 and one pregnant SHR $25.
† Unrelated to proposed project.
§ Salary support for principal investigator.

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Theodore A. Slotkin

Signature Theodore Slotkin Date

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Responsible officer of institution

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Title Vice President for Health Affairs

Signature W G Anlyan Date 6/11/74

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CURRICULUM VITAE

NAME:
Theodore Alan Slotkin

BORN: REDACTED

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EDUCATION AND DEGREES:
B.S. - Brooklyn College, CUNY, 1967
Ph.D. - Department of Pharmacology and Toxicology - University of Rochester, 1970

POSITIONS HELD:

June 1971 - present	Assistant Professor, Department of Physiology and Pharmacology, Duke University Medical Center
June 1970 - May 1971	Research Associate, Department of Biochemistry, Duke University Medical Center
February 1970 - May 1970	Postdoctoral Fellow, Department of Pharmacology and Toxicology, University of Rochester

SOCIETIES:

REDACTED

RESEARCH ACTIVITIES:
Neurochemistry
Neuropharmacology
Hypertension

BIBLIOGRAPHY

Slotkin, T.A. and DiStefano, V. Urinary metabolites of harmine in the rat and their inhibition of monoamine oxidase. Biochem. Pharmacol. 19:125-131 (1970).

Slotkin, T.A., DiStefano, V. and Au, W.Y.W. Blood levels and urinary excretion of harmine and its metabolites in man and rats. J. Pharmacol. Exp. Ther. 173:26-30 (1970).

Slotkin, T.A. and DiStefano, V. A model of harmine metabolism in the rat. J. Pharmacol. Exp. Ther. 174:456-462 (1970).

Slotkin, T.A. and DiStefano, V. Cardiovascular and respiratory effects of harmine. Proc. Soc. Exp. Biol. Med. 133:662-664 (1970).

Slotkin, T.A., Ferris, R.M. and Kirshner, N. Compartmental analysis of amine storage in bovine adrenal medullary granules. Mol. Pharmacol. 7:308-316 (1971).

Slotkin, T.A. and Kirshner, N. Uptake, storage and distribution of amines in bovine adrenal medullary vesicles. Mol. Pharmacol. 7:581-592 (1971).

Kirshner, N. and Slotkin, T.A. Stimulating experiences with the adrenal medulla. Proc. 8th Midwest Conf. on Endocrinol. and Metabolism. 95-113 (1972).

Slotkin, T.A. and Kirshner, N. All-or-none secretion of adrenal medullary storage vesicle contents in the rat. Biochem. Pharmacol. 22:205-219 (1973).

Slotkin, T.A. and Kirshner, N. Recovery of rat adrenal amine stores after insulin administration. Mol. Pharmacol. 9:105-116 (1973).

Slotkin, T.A. and Edwards, K. The effects of reserpine on the content and properties of rat adrenal medullary storage vesicles. Biochem. Pharmacol. 22:549-560 (1973).

Slotkin, T.A. Maturation of the adrenal medulla. I. Uptake and storage of amines in isolated storage vesicles of the rat. Biochem. Pharmacol. 22:2023-2032 (1973).

Slotkin, T.A. Maturation of the adrenal medulla. II. Content and properties of catecholamine storage vesicles of the rat. Biochem. Pharmacol. 22:2033-2044 (1973).

Slotkin, T.A. and Kirshner, N. Binding of amines to purified bovine adrenal medullary storage vesicle membranes. Biochem. Pharmacol. 22:2492-2497 (1973).

Slotkin, T.A. Hypothetical model of catecholamine uptake into adrenal medullary storage vesicles. Life. Sci. 13:675-683 (1973).

Green, H.O. and Slotkin, T.A. Reserpine-like effects of harmine on isolated adrenal medullary storage vesicles. Mol. Pharmacol. 9:748-755 (1973).

Kirshner, N. and Slotkin, T.A. Secretion and recovery of catecholamines by the adrenal medulla. Biochem. Pharmacol. in press.

Slotkin, T.A. and Green, H.O. Drug-resistant effect of adenine nucleotides and magnesium on catecholamine efflux from isolated adrenal medullary storage vesicles. Biochem. Pharmacol. in press.

Slotkin, T.A. Reserpine, in "Neuropoisons: Their Pathophysiological Actions" vol. 2 (Simpson, L.L. and Curtis, D.R., eds.). Plenum, in press.

Slotkin, T.A. Maturation of the adrenal medulla. III. Practical and Theoretical Considerations of Age-dependent Alterations in Kinetics of Incorporation of Catechol- and Non-catecholamines. Biochem. Pharmacol. in press.

Slotkin, T.A. and Green, H.O. Adrenal Medullary Storage Vesicles of the Spontaneously Hypertensive Rat. Biochem. Pharmacol. submitted.

Slotkin, T.A. Structure-Activity Relationships for the Reserpine-Like Actions of Derivatives of β-carboline in vitro. Life Sci., submitted.

Anderson, T.R. and Slotkin, T.A. Acute and Chronic Effects of Morphine on Rat Adrenal Medulla. Biochem. Pharmacol. submitted.

Mills, E. and Slotkin, T.A. Oxygen-Dependent Changes in Catecholamine Content of the Carotid Body. Science, submitted.

ABSTRACTS

Slotkin, T.A. and DiStefano, V. Urinary metabolites of harmine in the rat and their inhibition of monoamine oxidase. Fed. Proc. 28:797 (1969).

Slotkin, T.A., DiStefano, V. and Au, W.Y.W. Metabolism of harmine in rats and humans. Pharmacologist 11:273 (1969).

Slotkin, T.A. and DiStefano, V. A model of harmine metabolism in the rat. Fed. Proc. 29:678 (1970).

Slotkin, T.A. Efflux of ^{14}C-epinephrine from bovine adrenal medullary granules. Fed. Proc. 30:445 (1971).

Slotkin, T.A. and Kirshner, N. Structure-activity relationships for uptake and storage of amines by isolated bovine adrenal medullary vesicles. Pharmacologist 13:228 (1971).

Slotkin, T.A. and Kirshner, N. Depletion of rat adrenal medullary constituents following insulin. Fed. Proc. 31:521 (1972).

Slotkin, T.A. Uptake of epinephrine and metaraminol by isolated rat adrenal medullary vesicles following insulin administration. 5th Int. Cong. on Pharmacol. 217 (1972).

Slotkin, T.A. Uptake and storage of amines in isolated adrenal medullary vesicles of developing rats. Fed. Proc. 32:783Abs (1973).

Slotkin, T.A. Maturation of Adrenal Catecholamine Storage Vesicles of the Rat. Pharmacologist 15:210 (1973).

Kirshner, N. and Slotkin, T.A. Recovery of catecholamine stores following secretion by the adrenal medulla. Life. Sci. 13: lxxxiv-lxxxv (1973).

Slotkin, T.A. and Green, H.O. Adrenal Medullary Vesicles of Hypertensive Rats. Clin. Res. 22:13A (1974).

Anderson, T.R. and Slotkin, T.A. Acute and Chronic Effects of Morphine on Rat Adrenal Medulla. Fed. Proc. 33:511 (1974).

Anderson, T.R. and Slotkin, T.A. Effects of morphine on the adrenal medulla of developing rats. Pharmacologist in press.

Slotkin, T.A., Seidler, F.J. and Abou-Donia, M.D. Effects of tryptamines on epinephrine uptake into adrenal medullary vesicles. Pharmacologist in press.

13. Five most recent and pertinent articles:

1. T.A. Slotkin and H.O. Green. Adrenal Medullary Storage Vesicles of the Spontaneously Hypertensive Rat. Biochem. Pharmacol., submitted.

2. T.A. Slotkin. Maturation of the adrenal medulla. III. Practical and Theoretical Considerations of Age-dependent Alterations in Kinetics of Incorporation of Catechol- and Non-catecholamines. Biochem. Pharmacol., in press.

3. H.O. Green and T.A. Slotkin. Reserpine-like effects of harmine on isolated adrenal medullary storage vesicles. Mol. Pharmacol. 9:748 (1973).

4. T.A. Slotkin. Maturation of the adrenal medulla. II. Content and properties of catecholamine storage vesicles of the rat. Biochem. Pharmacol. 22:2033 (1973).

5. T.A. Slotkin. Maturation of the adrenal medulla. I. Uptake and storage of amines in isolated storage vesicles of the rat. Biochem. Pharmacol. 22:2023 (1973).

9. Details of experimental design

A. Introduction

The adrenal medulla is often utilized as a model of the sympathetic neuron; both tissues arise embryonically from the neural crest and both have the ability to synthesize, store and secrete catecholamines. Each contains storage vesicles which can accumulate amines by a mechanism which is stimulated by ATP-Mg^{2+} and blocked by reserpine. The vesicles contain dopamine beta-hydroxylase (DBH), chromogranins and adenine nucleotides as well as catecholamines; it is accepted generally that the catecholamines and adenine nucleotides (primarily ATP) form a storage complex in a molar ratio of 4 to 1.

During prenatal and postnatal development, there is a marked increase in catecholamine levels in adrenergic neurons and in the adrenal medulla (1-5), as well as changes in catecholamine synthesizing enzymes (1,6). Although the necessary enzymes are present early in gestation, catecholamines do not appear until late in gestation, at a time when storage vesicles first become detectable (7,8), suggesting that the storage vesicles play a determining role in the increase in adrenal amines. Consequently, the largest changes in catecholamine content occur in the postnatal period (0-6 weeks after birth). Spontaneously hypertensive Wistar rats (SHR) first show significantly elevated blood pressures towards the end of this period (5 weeks), along with disturbances in sympathetic catecholamine synthesis, storage and release (9). Similarly, in studies utilizing uninephrectomized rats treated with desoxycorticosterone acetate and NaCl, it has been shown that the resultant hypertension is accompanied by a defect in catecholamine storage such that cardiac storage vesicles become "leaky"(10). Westfall(11) has likewise demonstrated changes in catecholamine turnover in rats with elevated systolic blood pressures produced by chronic nicotine administration. Impairment of catecholamine storage has been implicated in essential hypertension in humans, as evidenced by increases in excretion of catecholamines and their metabolites in individuals with that disease (12).

In each case, hypertension was accompanied by a disturbance in sympathetic function possibly involving impaired storage. Therefore, it is important to examine systematically the properties of the storage vesicles in at least one of the model systems. The SHR is probably the most reliable of all methods of hypertension to use for a study of this type: hypertension develops rapidly and requires no pharmacological or surgical intervention, the animals are available as a genetically pure strain, and inbred normotensive Wistar rats provide a valid control (9). It is of additional interest that, although the mechanism of hypertension may be different from SHR, essential hypertension in humans is probably genetic in origin (13).

While it is of obvious importance to determine the mechanisms involved in the age-dependent increases in sympatho-adrenal amines and the development of altered storage in SHR, few studies have been published on the properties of the storage vesicles during these changes. Brundin (14) measured the rate of efflux of endogenous

amines from isolated neonatal rabbit adrenal vesicles, but did not do simultaneous studies utilizing vesicles from adults; furthermore, he reported only two time points (30 and 60 minutes), while several studies have shown that amine efflux is biphasic and therefore requires kinetic analysis of many time points to obtain a valid picture of amine storage (15-19). Mirkin (5) has studied the storage properties of vesicles from fetal and developing rat heart; these studies utilized measurements of in vivo uptake or uptake in the isolated, perfused heart. Thus, the observed development of uptake or storage might have reflected proliferation of adrenergic neurons in the heart, or changes in the uptake of amines across the axonal membrane, rather than alterations in the storage vesicles per se. The studies by Mirkin (5) also indicate the difficulties of working with adrenergic neurons compared to the adrenal medulla; a single density gradient experiment required the pooling of 50-60 fetal or neonatal rat hearts. Obviously, experiments of this type are not feasible if large numbers of observations need to be made. For this reason, the adrenal medulla provides a more useful model than sympathetic nerves with which to study amine storage. Purified adrenal vesicles can be obtained in high yield by a relatively rapid discontinuous density gradient technique (20), while much lower yields of comparably purified sympathetic nerve vesicles are obtained after more lengthy procedures. Furthermore, the high concentration of storage vesicles in the adrenal permits the evaluation of properties which would be far more difficult to determine in nerve vesicles.

Similarly, because of the difficulty in evaluating the properties of nerve storage vesicles, most of the studies on catecholamine turnover in hypertension have involved whole animal or whole organ studies (9-13). There is, however, one report of altered cardiac vesicular amine storage in which elevated ratios of extragranular to intragranular amines were observed in hypertensive rats (10). These data further reinforce the need for studies which can identify specific alterations in vesicle properties.

For the past four years, the research of this investigator has been concerned with the development of sensitive and appropriate methods for the evaluation of the properties of the catecholamine storage vesicles of the adrenal medulla (16-19). By using these techniques, the following parameters can be measured:

a. Uptake and storage capabilities of the vesicles. The simultaneous measurement of the accumulation of radioactively labelled amines by the vesicles along with the efflux of endogenous and labaled amines permits evaluation of these two parameters. The rate of efflux is determined by the stability of storage, while the accumulation is a measure of storage stability and affinity for uptake. Additionally, the relative importance of ATP-Mg^{2+} stimulated uptake can be evaluated by measuring the accumulation of metaraminol, an amine which is incorporated by a primarily ATP-Mg^{2+}-independent mechanism (16, 17).

b. Concentrations of intravesicular components. Vesicles are purified by discontinuous sucrose density gradient centrifugation. The subcellular distributions of catecholamines, ATP and DBH can thus be readily determined, along with the fragility of the vesicles. The buoyant density of the vesicles can also be studied by continuous density gradient centrifugation, which provides a sensitive measure for evaluating small differences in the densities of different populations of vesicles.

c. Secretion and recovery of amines and vesicles. This can be evaluated using neurogenic secretion evoked by insulin-induced hypoglycemia or non-neurogenic amine loss produced by reserpine.

Using these techniques, work supported by the Council for Tobacco Research has been conducted by the investigator over the last 18 months on the development of the adrenal medulla in NWR and on the alterations in catecholamine disposition in adult SHR. The results have been summarized in detail in the progress reports and in publications from this project (see list), but a brief listing appears below:

1. The maturation process is accompanied by profound changes in the uptake, storage, synthesis and release of adrenomedullary catecholamines; various markers have been found to study the process in the projected studies for SHR.

2. Adult SHR show an adrenal catecholamine biochemical pattern typical of chronic understimulation; however the sympatho-adrenal axis is hyperresponsive to ordinarily mild stress stimuli.

3. Alterations in the storage vesicle membrane of the SHR result in abnormal responses to antihypertensive drugs and may alter the effects of agents which increase catecholamine turnover, such as nicotine.

In the proposed extension of this study, we intend to determine in detail:

1. The effects of chronic and acute nicotine administration in NWR and SHR.

2. The effects of chronic and acute administration of antihypertensive agents.

3. The time course of development of hypertension and changes in catecholamine disposition during maturation of the SHR.

These studies will identify whether sympatho-adrenal changes cause or are caused by the changes in cardiovascular dynamics and will demonstrate whether drugs which affect catecholamine disposition can improve or worsen the defects in the SHR.

B. Methods

In studies with antihypertensive drugs, adult SHR and NWR will be given single or repeated injections of reserpine, α-methyldopa (Aldomet), DBH inhibitors (disulfiram and fusaric acid), α-methyl-p-tyrosine and 6-hydroxydopamine (acute only). Appropriate doses for acute and chronic administration of all of these agents have already been worked out and published in our and other laboratories. In some experiments, chlorisondamine, a long-acting ganglionic blocking agent, will be administered to interrupt sympathetic reflex activity and enable studies of direct actions of each drug. SHR and NWR will be sacrificed at intervals of 4 and 24 hr after initial administration, at intervals of several days during chronic administration, and several weeks after acute administration of 6-hydroxydopamine.

Studies with nicotine will involve acute and chronic (twice daily) doses of 2.5 mg/kg s.c. and 25 mg/kg s.c. for periods up to 2 months; animals will be sacrificed at weekly intervals.

Studies with SHR neonates will involve sacrifice at 10-day intervals from birth to 50 days of age; blood pressure will be monitored by tail plethysmography (20). In some studies, SHR and NWR neonates will be chemically sympathectomized with 6-hydroxydopamine and subsequent changes in blood pressure and catecholamine disposition will be monitored.

After sacrifice, adrenals will be removed, cleaned of connective fat and tissue, and homogenized in isotonic sucrose. Subcellular fractions will be obtained by differential and density gradient centrifugation. The detailed methodologies will not be described here because they appeared in our previously funded CTR applications; for this information, please refer to the appended reprints and manuscripts. We will make the following determinations:

1. Activities of catecholamine biosynthetic and degradative enzymes (tyrosine hydroxylase, dopa decarboxylase, dopamine β-hydroxylase and monoamine oxidase).

2. Catecholamine levels in the whole tissue.

3. Subcellular distributions of catecholamines, ATP and dopamine β-hydroxylase.

4. Buoyant density and fragility of storage vesicles.

5. Ability of storage vesicles to incorporate ^{14}C-epinephrine or ^{3}H-metaraminol in terms of:

a. Uptake capacity per gland.
b. Uptake capacity per vesicle.
c. Affinity and maximal transport rate for each amine.

6. Efflux of endogenous and newly-incorporated amines.

7. Functional rate of β-hydroxylation in intact vesicles.

These data will enable us to identify specific changes in catecholamine disposition in terms of hypertension or drug-induced changes in synthesis, uptake, storage or secretion in a fashion analogous to our previous work in developing NWR and adult SHR, and to establish whether the actions of nicotine and antihypertensives differ in the SHR compared to NWR.

C. Rationale and significance.

The development of hypertension in spontaneously hypertensive rats, in rats with surgically or pharmacologically induced hypertension, in human essential hypertension, and in rats chronically treated with nicotine, is associated with alterations in catecholamine disposition. The sympathetic nervous system and its endocrine counterpart, the adrenal medulla, exert important regulatory functions on the entire cardiovascular system. During the first six weeks after birth, the adrenergic neurons and adrenal medulla of the rat undergo marked changes in catecholamine synthesis, uptake, storage and release. At the same time, hypertension begins to develop in spontaneously hypertensive Wistar rats, which is associated with defects in the physiological disposition of sympathetic amines. The proposed study is designed to identify at what time after birth these changes occur. The earlier studies from our lab have shown that some of the sites of action of antihypertensive drugs and nicotine are altered in the SHR. The proposed studies will identify the differences in the actions of these agents in SHR and NWR, which could be of considerable significance in evaluating the potential exacerbation of sympatho-adrenal alterations by nicotine as well as the development of refractoriness to antihypertensive drugs.

D. References.

1. L.J. Ignarro and F.E. Shideman. J. Pharmacol. Exp. Ther. 159: 38 (1968).
2. F.W. Heggeness, J. Diliberto and V. DiStefano. Proc. Soc. Exp. Biol. Med. 133:1413 (1970).
3. L.L. Iversen, J. de Champlain, J. Glowinski and J. Axelrod. J. Pharmacol. Exp. Ther. 157:509 (1967).
4. B. Hökfelt. Acta Physiol. Scand. 25:Suppl. 92 (1951).
5. B.L. Mirkin. Fed. Proc. 31:65 (1972).
6. R.L. Patrick and N. Kirshner. Dev. Biol. 29:204 (1972).
7. S. Daikoku, O. Takashi, A. Takahashi and M. Sako. Tokushima J. Exp. Med. 16:153 (1969).

8. L.G. Elfvin. Ultrastructures 17:45 (1967).
9. W.J. Louis, R. Tabei, S. Spector and A. Sjoerdsma. Circ. Res. 24:Suppl. 1, 93 (1969).
10. J. de Champlain, L. Krakoff and J. Axelrod. Circ. Res. 24: Supply. 1, 75 (1969).
11. T.C. Westfall. Eur. J. Pharmacol. 10:19 (1970).
12. A.W. Stott and R. Robinson. Clin. Chim. Acta 16:249 (1967).
13. M. Mendlowitz, R.L. Wolf and S.E. Gitlow. Am. Heart J. 79: 401 (1970).
14. T. Brundin. Acta Physiol. Scand. 66:406 (1966).
15. F. Lishajko. Acta Physiol. Scand. 79:64 (1970).
16. T.A. Slotkin, R.M. Ferris and N. Kirshner. Mol. Pharmacol. 7:308 (1971).
17. T.A. Slotkin and N. Kirshner. Mol. Pharmacol. 7:581 (1971).
18. T.A. Slotkin and N. Kirshner. Biochem. Pharmacol. 22:205 (1973).
19. T.A. Slotkin and N. Kirshner. Mol. Pharmacol. 9:105 (1973).
20. J.R. Williams, T.R. Harrison and A. Grollman. J. Clin. Invest. 18:373 (1939).